PATENT COOPERATION TREATY

02. Dez. 2004

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From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	01.12.2004
Applicant's or agent's file reference: Case 21409 WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP 03/10489	22.09.2003	27.09.2002

Applicant: DSM IP ASSETS B.V. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the International preliminary examining authority:

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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Case 21409 WO	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/10489	International filing date *(day/month/year)* 22.09.2003	Priority date *(day/month/year)* 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12P17/04		
Applicant DSM IP ASSETS B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.03.2004	01.12.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Gruber, A Telephone No. +31 70 340-8997

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/10489

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-10 as originally filed

Claims, Numbers

1-10 received on 31.08.2004 with letter of 25.08.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☒ contained in the international application in written form.
- ☒ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-10
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-10
	No:	Claims	

2. Citations and explanations

see separate sheet

The present application provides the use of enzyme B of Gluconobacter oxydans DSM 4025 in a process for producing L-ascorbic acid, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone or L-galactonic acid from various substrates.

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: EP-A-0832974
D2: EP-A-0911415
D3: US-B1-6242233
D4: Applied And Environmental Microbiology, Washington,DC, US (1997), 63(2), 454-460

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 The subject-matter of claims 1-10 is new in the sense of Article 33(2) PCT and is susceptible to industrial application (Article 33(4) PCT).

2 The subject-matter of claims 1, 2, 5 - 8 (claims 5 - 7 where relating to claims 1, 2) involves an inventive step in the sense of Article 33(3) PCT.

2.1 The document D1, which is considered to represent the closest prior art, discloses (the references in parentheses applying to this document): a process for the production of 2-keto-L-gulonic acid (table 10; claims 16, 18; examples 8 - 11) with an enzyme having the amino acid sequence of SEQ ID NO:2 isolated from Gluconobacter oxydans DSM 4025 (SEQ ID NO 8, claim 1) and functional derivatives thereof (claim 1).

The difference, in terms of the claimed technical features, between the subject-matter of claims 1, 2, 5 - 8 of the present application on the one hand and the closest prior art document D1 on the other, is the use of enzyme B having the amino acid sequence of SEQ ID NO:2 for the production of L-ascorbic acid from suitable precursors in one step.

The objective technical problem underlying the subject-matter of the present

application is therefore the provision of a further use of enzyme B for the production of L-ascorbic acid.

The solution to this problem proposed by claims 1, 2, 5 - 8 consists of the provision of substrates for enzyme B for the production of L-ascorbic acid in one step.

The enzyme described in document D1 having the amino acid sequence of SEQ ID NO:2 has broad substrate specificity (table 10). For example, L-idose was converted by this enzyme to L-idonic acid (table 10).
Document D1 suggests using the enzyme having the amino acid sequence of SEQ ID NO:2 for the production of the L-ascorbic acid precursor 2-keto-L-gulonic acid (table 10), aldehyde(s), carboxylic acid(s) and ketone(s) from a corresponding substrate (table 10; claims 15, 17).
The involvement of an enzyme having the amino acid sequence of SEQ ID NO:2 in the production pathway of L-ascorbic acid is already known from the teaching of document D1 which discloses the conversion of L-Sorbosone to 2-keto-L-gulonic acid (table 10), which is an important intermediate for the production of L-ascorbic acid and can be transformed into L-ascorbic acid by a method know in the state of the art (claim 19).

In addition, document D2 describes the production of L-ascorbic acid by Gluconobacter oxydans DSM 4025 using L-gulono-gamma-lactone as substrate (examples 2, 3).

However, neither document D1 nor document D2 discloses the use of substrates for enzyme B for the production of L-ascorbic acid in one step.

Thus, the subject-matter of claims 1, 2, and 5 - 8 (claims 5 - 7 where relating to claims 1, 2) involves an inventive step and satisfies the criterion set forth in Article 33(3) PCT.

2.2 The subject-matter of claims 3 - 7 (claims 5 - 7 where referring to claims 3, 4) 9, and 10 is inventive (Article 33(3) PCT).

Claims 3 - 7, 9, and 10 refer to the use of enzyme B of Gluconobacter oxydans DSM 4025 in a process for producing L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone or L-galactonic acid from various substrates.

The document D1, which is considered to represent the closest prior art, discloses (the references in parentheses applying to this document): an enzyme having the amino acid sequence of SEQ ID NO:2 isolated from Gluconobacter oxydans DSM 4025 (SEQ ID NO 8, claim 1) that has alcohol and/or aldehyde dehydrogenase activities (page 2, lines 1 - 2).

The difference, in terms of the claimed technical features, between the subject-matter of claims 3 - 7, 9, and 10 of the present application on the one hand and the closest prior art document D1 on the other, is the substrate used and the products.

The objective technical problem underlying the subject-matter of the present application is therefore the production of L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone or L-galactonic acid using an enzyme having the amino acid sequence of SEQ ID NO:2.

The solution to this problem proposed by the present application consists of the conversion of L-gulose to L-gulono-1,4-lactone and L-gulonic acid and of L-galactose to L-galactono-1,4-lactone and L-galactonic acid using an enzyme having the amino acid sequence of SEQ ID NO:2.

Document D1 does not suggest -neither alone nor in combination with any other available document- to use L-gulose and L-galactose as a substrate for an enzyme having the amino acid sequence of SEQ ID NO:2 in order to produce L-gulono-1,4-lactone and L-gulonic acid and L-galactono-1,4-lactone and L-galactonic acid, respectively.

Thus, the subject-matter of claims 3 - 7 (claims 5 - 7 where referring to claims 3, 4), 9, and 10 involves an inventive step and satisfies the criterion set forth in Article 33(3) PCT.

3 Aside from the above-mentioned remarks, the following objections are made:

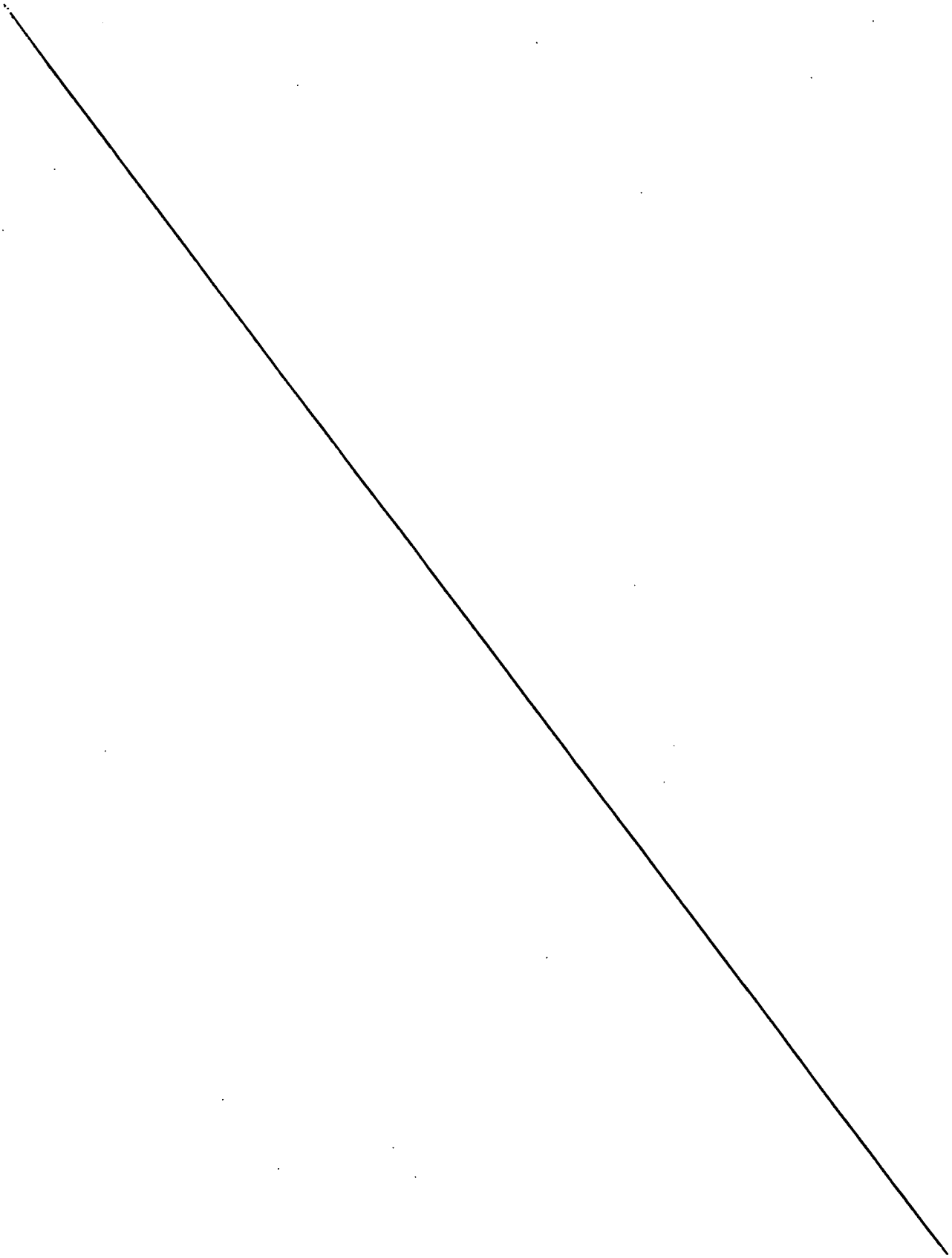
3.1 The term "about" used in claims 6 - 10 is vague and unclear and leaves the reader in doubt as to the meaning of the technical features to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Article 6 PCT).

3.2 The application does not meet the requirements of Article 6 PCT, because claims 1 - 4, and 8 - 10 do not sufficiently define the subject-matter in terms of technical

features of the invention.
Document D1 discloses that an isolated enzyme having the amino acid sequence of SEQ ID NO: 2 uses L-idose as substrate to produce L-idonic acid but not L-ascorbic acid. Document D4 discloses that E. coli JM109, the organism used in examples 1 - 4 of the present application, itself is capable of producing 2-keto-L-gulonate (page 455, right-hand column, paragraph 4; table 1).
The present application discloses only the production of L-ascorbic acid in E. coli JM109 transformed with a gene encoding for an enzyme having the amino acid sequence of SEQ ID NO: 2. It appears that the process for L-ascorbic acid production described in the present application depends on the presence of E. coli JM109 or other additives. Therefore, the present application does not disclose how to produce L-ascorbic acid by using only the isolated enzyme having the amino acid sequence of SEQ ID NO: 2. It seems that additional technical features, e.g. the use of E. coli JM109, besides an enzyme having the amino acid sequence of SEQ ID NO: 2 are necessary to arrive at the claimed products.
Since independent claims 1 - 4, and 8 - 10 do not contain these features, they do not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3 (b) (I), (ii) PCT that any independent claim must contain all the technical features essential to the definition of the invention.
Furthermore, the subject-matter of claims 1 - 4, and 8 - 10 is insufficiently disclosed (Article 5 PCT).

3.3 Examples 1 and 3 of the present application describe the production of L-ascorbic acid from L-gulose and L-galactose when given as substrate to E. coli JM109 transformed with said enzyme. However, the present application shows no evidence that the experimental setup described for the production of L-ascorbic acid from L-gulose and L-galactose is also feasible for the production of L-ascorbic acid from the other substrates mentioned in claims 1, 2, and 8, especially since this might involve multiple steps. Therefore, the application does not meet the requirement of Article 5 PCT, since the invention is not disclosed in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art over the whole scope claimed.

3.4 Claims 8, 9, and 10 define enzyme B of G. oxydans DSM 4025 by its parameters. The definition is not clear according to Article 6 PCT. Characterization of a product mainly by its parameters is only allowed in those cases where the invention cannot be adequately defined in any other way. In this instance, however, such a formulation is not allowable because it appears possible to define the subject-matter in more concrete terms, i.e. by its amino acid sequence SEQ ID NO: 2.



1. A process for the production of L-ascorbic acid comprising: (94)

(a) contacting an enzyme with a substrate which is selected from the group consisting of L-gulose, L-galactose, L-idose, and L-talose; and

(b) isolating L-ascorbic acid from the reaction mixture,

wherein said enzyme has the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto with the activity to produce L-ascorbic acid.

2. A process for the production of L-ascorbic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, with the activity to produce L-ascorbic acid, whereby L-ascorbic acid is produced from a substrate which is selected from the group consisting of L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, L-galactonic acid, L-idono-1,4-lactone, L-idonic acid, L-talono-1,4-lactone, and L-talonic acid.

3. A process for the production of L-gulono-1,4-lactone or L-gulonic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, with the activity to produce L-gulono-1,4-lactone or L-gulonic acid, whereby L-gulono-1,4-lactone or L-gulonic acid is produced from L-gulose.

4. A process for the production of L-galactono-1,4-lactone or L-galactonic acid with an enzyme having the amino acid sequence of SEQ ID NO: 2 or an amino acid sequence that is 90% identical thereto, with the activity to produce L-galactono-1,4-lactone or L-galactonic acid, whereby L-galactono-1,4-lactone or L-galactonic acid is produced from L-galactose.

5. A process according to any one of claims 1 to 4 comprising (a) contacting the enzyme with the respective substrate and (b) isolating the product which is selected from the group consisting of L-ascorbic acid, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, and L-galactonic acid from the reaction mixture.

6. A process according to any one of claims 1 to 5, wherein the process is conducted for 1 to 120 h at a pH of about 1 to about 9 and at a temperature of about 13°C to about 45°C.

7. A process according to claim 6, wherein the process is conducted at a pH of about 2 to about 8 and at a temperature of about 18°C to about 42°C.

8. Use of Enzyme B of *G. oxydans* DSM 4025 in a process for producing L-ascorbic acid from a substrate which is selected from the group consisting of L-gulose, L-galactose, L-idose, L-talose, L-gulono-1,4-lactone, L-gulonic acid, L-galactono-1,4-lactone, and L-galactonic

acid; wherein Enzyme B has the following physico-chemical properties:

(a) molecular weight of about 60,000 Da on SDS-PAGE;

(b) substrate specificity for primary and secondary alcohols and aldehydes;

(c) pH-stability at pH of about 6 to about 9;

(d) pH-optimum at pH of about 8.0; and

(e) inhibited by Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+}.

9. Use of Enzyme B of *G. oxydans* DSM 4025 in a process for producing L-gulono-1,4-lactone or L-gulonic acid from L-gulose, wherein Enzyme B has the following physico-chemical properties:

(a) molecular weight of about 60,000 Da on SDS-PAGE;

(b) substrate specificity for primary and secondary alcohols and aldehydes;

(c) pH-stability at pH of about 6 to about 9;

(d) pH-optimum at pH of about 8.0; and

(e) inhibited by Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+}.

10. Use of Enzyme B of *G. oxydans* DSM 4025 in a process for producing L-galactono-1,4-lactone or galactonic acid from L-galactose, wherein Enzyme B has the following physico-chemical properties:

(a) molecular weight of about 60,000 Da on SDS-PAGE;

(b) substrate specificity for primary and secondary alcohols and aldehydes;

(c) pH-stability at pH of about 6 to about 9;

(d) pH-optimum at pH of about 8.0; and

(e) inhibited by Cu^{2+}, Zn^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+}.